PB84-109990

Effects of Suspended Clay on Bioconcentration of Kelthane (Trade Name) in Fathead Minnows

(U.S.) Environmental Research Lab.-Duluth, MN

24 Jan 83

U.S. Department of Commerce:
National Technical Information Service
NTIS

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/J-83-069	2.
3. RE PB84-109990	
4. TITLE AND SUBTITLE: Effects of Suspended Clay on Bioconcentration of Kelthane® in Fathead Minnows (Journal Version)	5. REPORT DATE 1983
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S): J. G. Eaton, V. R. Mattson, L. H. Mueller, and D. K. Tanner	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS: U.S. Environmental Protection Agency, Environmental Research Laboratory-Duluth, 6201 Congdon Boulevard, Duluth, Minnesota 55804	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS: Same as above	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE EPA/600/03

15. SUPPLEMENTARY NOTES: Arch. Environ. Contam. Toxicol. 12, 439-445 (1983)

16. ABSTRACT

The experiment was designed to determine the effect of suspended clay sediments on the bioconcentration of the chlorinated hydrocarbon pesticide Kelthane® (dicofol, 4-chloro-α-(4-chlorophenyl)-α-(trichloromethyl)benzene methanol) in fathead minnows (Pimephales promelas). The test was carried out under continuous-flow conditions with a modified Mount-Brungs toxicant dilution apparatus and an exposure period of 28 days. Two known safe Kelthane® treatment levels were tested in both filtered lake water and lake water containing approximately 65 mg/L of clay particles. The clay sediments decreased bioconcentration in fathead minnows by 35%, indicating that the 30+% of total Kelthane® sorbed onto clay was biologically unavailable to the fish. Bioconcentration factors at the two Kelthane® concentrations were not significantly different. Steady-state concentrations were estimated to occur in 39 to 60 days of exposure at 9,500 to 18,900 times water levels.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT: RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES 8
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

REPRODUCED BY NATIONAL TECHNICAL INFORMATION SERVICE U.S. DEPARTMENT OF COMMERCE SPRINGFIELD, VA 22161

EPA-600/J-83-069
JOURNAL ARTICLE

Arch. Environ. Contam. Toxicol. 12, 439–445 (1983)

Archives of Environmental Contamination and Toxicology

Effects of Suspended Clay on Bioconcentration of Kelthane® in Fathead Minnows

John G. Eaton, Vincent R. Mattson, Leonard H. Mueller, and Danny K. Tanner

Environmental Research Laboratory—Duluth, Office of Research and Development, U.S. Environmental Protection Agency, 6201 Congdon Boulevard, Duluth, Minnesota 55804

Abstract. The experiment was designed to determine the effect of suspended clay sediments on the bioconcentration of the chlorinated hydrocarbon pesticide Kelthane® (dicofol, 4-chloro-α- (4-chlorophenyl)-α-(trichloromethyl) benzene methanol) in fathead minnows (*Pimephales promelas*). The test was carried out under continuous-flow conditions with a modified Mount-Brungs toxicant dilution apparatus and an exposure period of 28 days. Two known safe Kelthane® treatment levels were tested in both filtered lake water and lake water containing approximately 65 mg/L of clay particles. The clay sediments decreased bioconcentration in fathead minnows by 35%, indicating that the 30 + % of total Kelthane® sorbed onto clay was biologically unavailable to the fish. Bioconcentration factors at the two Kelthane® concentrations were not significantly different. Steady-state concentrations were estimated to occur in 39 to 60 days of exposure at 9,500 to 18,900 times water levels.

This study was the first in a series using bioconcentration as a means of determining the influence of different types of suspended particulates on the biological availability of pesticides. Most toxicity and bioconcentration tests are conducted in "clean" water with low suspended solid concentrations and often little is known about how such test results relate to natural systems where suspended solids of a diverse nature are common. Several investigators have used acute mortality tests to determine the effect of particulates on biological activity (Ferguson *et al.* 1965; Brungs and Bailey 1967). For chemicals which bioconcentrate, measurement of tissue residues offers some advantages over acute mortality tests, because only one or two test concentrations are required, fewer test organisms are usually employed, and a specific point estimate (LC50 value) is not required. Also, it provides greater flexibility in selecting exposure concentrations representative of those occurring under natural circumstances. Disadvantages are that information related to safe concentrations is required before starting a bioconcentration test, techniques must be available for measuring tissue as well as water concentrations of the test material, and the test might last longer than four days. However, recent developments in bioconcentration test design procedures are reducing exposure time by improving the predictability of the time required to reach steady-state. The ability to estimate steady-state tissue concentrations from exposures of shorter duration is also improving.

The present study was designed to determine the effect of a predominantly inorganic suspended particulate on the bioconcentration of Kelthane® (dicofol, 4-chloro-α-(4-chlorophenyl)-α-(trichloromethyl) benzene methanol) by fathead minnows (*Pimephales promelas*). Bioconcentration is defined as the accumulation of Kelthane® from water into or onto whole fish tissue. This chemical is used as a miticide on vegetables, fruits, grain, and ornamental crops. It was selected for study as a representative of the chlorinated hydrocarbon category of pesticides, and in response to the U.S. Environmental Protection Agency, Office of Pesticides Programs interest in its bioconcentration and sorption properties.

Materials and Methods

Exposure System

The exposure system consisted of two adjacent modified proportional diluters (Mount and Brungs 1967) that delivered one L

of test water to each exposure tank per cycle. The diluent water for both diluters was filtered Lake Superior water with nearly complete removal of suspended solids. This was accomplished with a sand filter, a Filterite® 5 μm bleached white cotton filter, and finally, a Nuclepore® 0.2 μm "QR" membrane filter. One diluter produced two concentrations of Kelthane® (nominally 12.5 and 1.25 μg/L) plus a control using filtered diluent water, while the other used a suspended clay diluent to produce the same two concentrations of Kelthane® plus a clay-water control. Flow splitting cells (Benoit and Puglisi 1973) delivered the Kelthane® concentrations and control waters to duplicate 57 L all-glass aquaria (61.0 × 30.5 × 30.5 cm). Water depth was maintained at a minimum of 15 cm. All tanks were gently aerated to keep the clay particles in suspension. The time for replacement of water equal to one tank volume was approximately two hr. Fluorescent lamps provided a 16-hr light and 8-hr dark photoperiod.

Two 19-L stainless steel soda carbonation cans, of the type used in automatic vending machines, were used to prepare the toxicant solution (Phipps 1982). Kelthane® was thinned from the heavily viscous technical grade form with acetone and painted onto 10 mesh stainless steel screen. The screen was formed into a column 10 cm in diameter and 50 cm high, the acetone was evaporated, and, finally, the screen was placed in the first "pop can". A calibrated metering pump delivered a desired amount of diluent water to the top of the first can during each diluter cycle. The same amount of toxicant-bearing water exited the second can for the diluter by way of an outlet tube located within a few mm of the bottom.

Water chemistry measurements were made at least once a week in duplicate tanks during the exposure period. Dissolved oxygen concentrations during the test ranged from 7.9 to 9.1 mg/L. The ranges for total hardness and total alkalinity were 45–52 mg/L and 42–51 mg/L, respectively. The pH measurements ranged from 7.40 to 7.65. Water temperature was maintained at 23 ± 2°C.

Red clay was obtained from the south shore of Lake Superior, transported to the laboratory in polyethylene garbage containers and stored at 10°C. Because this soil exists in undisturbed deposits resulting from the most recent glaciation of the area, it contains very low amounts of organic matter. The mineral content of the <2 μm fraction, in decreasing order of prevalence is quartz, calcite, kaolinite, illite, montmorillonite, plagioclase, a minor unknown clay, minor amphibole, and very minor talc (Marklund *et al.* 1981). Prior to use, filtered Lake Superior water was added to a storage container and mixed with clay. The mixture was decanted and fractionated by sedimentation for 24 hr to remove particles larger than 2 μm settling size (Jackson 1956). The mixture containing the 2 μm and smaller clay particles was transferred to a 200 L fiberglass recirculation reservoir maintained at 10°C from which a peristaltic pump metered the mixture to a headbox which fed the clay diluter. Concentrations of suspended particles were monitored daily with turbidity measurements taken in the diluter system headbox and each duplicate tank. The samples were taken at mid-tank level in each system and measured with a Hack model 2100 A turbidimeter. The test tank mean nephelometric turbidity unit (NTU) level for the 28 day exposure was 80.1 (sd = 7.6, n = 69). Weight analysis of several samples after filtration at 0.45 μm indicated 1 NTU to equal 0.82 mg of clay per L of water. Sorption equilibrium of Kelthane® on clay occurred rapidly; the amount sorbed 30 hr after mixing was no greater than that sorbed after 2 hr.

The Rohm and Haas Company supplied technical grade Kelthane® (Lot #1906, 87% purity) for the study. An analysis for specific contaminants revealed that this lot contained 0.35% *p,p'* DDE.

Biological Procedures

Fathead minnows were obtained from cultures maintained at the Environmental Research Laboratory—Duluth. Twenty-five, 41-day-old fish were placed in each of the twelve tanks at the beginning of the test. The fish were fed frozen brine shrimp once daily during the exposure period. Groups of five fish were removed from each tank at five sampling times during the 28-day exposure. These and other biological test methods generally followed the procedure recommended by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) and the Proposed Method for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Molluscs (Stephan and Schimmel unpublished manuscript, ERL—Duluth). Previous toxicity studies with Kelthane in Lake Superior water had demonstrated a 96-hr LC50 of 510 μg/L (Holcombe *et al.* 1982) and a no-effect, 30-day early-life-stage test concentration of 19 μg/L (Spehar *et al.* 1982).

Analytical Methods

During the exposure test, waters were analyzed for Kelthane® at least once a week in duplicate chamber samples siphoned from mid-depth. One hundred seventy five ml of sample was placed in a 200 ml volumetric flask containing 25 ml of hexane and mixed vigorously on a magnetic stirrer for 30 min. One ml of this extract was analyzed for Kelthane® on a Hewlett Packard 5710 gas chromatograph with automatic sampler and Ni^{63} electron capture detector. A 1.8 m by 2 mm (ID) glass coil substrate column contained 4% SE-30 and 6% OV-210 on 80/100 mesh Gas Chrom Q. The carrier gas was argon containing 5% methane; column temperature was 195°C. Recovery of Kelthane® spiked into four water samples at 0.04 μg/ml averaged 93.5% (sd = 3.4%). Instrument variability was calculated to be 4.4% and the detection limit of Kelthane® in water by this technique was 0.1 μ/L.

At each sampling interval, five fish were indiscriminately netted from each test tank, weighed, and stored frozen in aluminum foil packets prior to analysis. After rinsing with hexane, the five-fish composite from each tank was blended for 2 min with 100 ml hexane and 5 g Na_2SO_4. The sample was placed on a 4 g Florisil® column and eluted with 50 ml of a 6% ethyl ether, 94% petroleum ether mixture. The sample was concentrated and diluted to volume for GC analysis under the same conditions as the water samples. Quality control recoveries from nine Florisil® column samples spiked with 6.8 μg Kelthane® averaged 99% (sd = 5%). All tissue concentrations of Kelthane® were greater than 2 μg/g; the detection limit for tissues was 0.01 μ/g. Fish residue concentrations were calculated on a whole-body, wet-weight basis.

The technical grade Kelthane® contained both ortho-para (*o,p*) and para-para' (*p,p'*) isomers in a ratio of 1 to 3.55. Only the *p,p'* isomer was accumulated to any extent in tissues and, therefore, all bioconcentration factors (BCF) were calculated on the basis of *p,p'* determinations in water and fish.

After the bioconcentration test was completed, a series of analyses was conducted to approximate the amount of Kelthane® sorbed onto clay at the high exposure level in the bioconcentration test. Kelthane® was spiked into a stirred clay suspension 2 ½ hr before treatment, because this is the estimated time for replacement of 50 to 75% of the water in test tanks and is representative of the water to clay partitioning experienced by test fish. Separation of clay and water was achieved by centrifuga-

Table 1. Replicate (A and B) Kelthane® bioconcentration factors at five exposure intervals and two test conditions for fathead minnows

Exposure intervals (days)	High Kelthane® concentration				Low Kelthane® concentration			
	12.38 μg/L[a] No clay		14.95 μg/L Clay		1.15 μg/L No clay		1.33 μg/L Clay	
	A	B	A	B	A	B	A	B
2	1600	—	1200	1500	1600	1700	1400	1100
4	3600	3100	2400	1900	3900	2800	2900	2600
8	7500	6300	6000	4900	7500	8000	5300	4700
16	9700	8900	6700	—	10500	10500	8300	8300
28	10500	14500	8700	7400	13900	13100	9800	11300

[a] Measured mean water concentrations that were used in BCF calculations

$$\left(\text{BCF} = \frac{\text{tissue concentration}}{\text{water concentration}}\right)$$

tion. The efficiency of the process was determined by weighing the residues remaining after passage of centrifuged and unmodified aliquots through a 0.45 μm cellulose acetate filter. Since initial samples spun at 2,600 rpm were found to retain 19.7% (by weight) of the solids in suspension, centrifugation was increased to 22,000 rpm for 30 min. at 10°C which left only 1.3 mean percent of the filterable solids in the water fraction.

The supernatant from two centrifuge tubes (~37 ml/tube) was combined and extracted with hexane (3:1) for 60 min on a magnetic stirrer. An acetonitrile-hexane (1:4) extraction procedure was used to remove Kelthane® from the centrifuged clay pellet after decanting the supernatant. The tube contents were stirred on a vortex mixer before transfer to a separatory funnel and acetonitrile removal. The sample was washed with distilled water, dried with Na_2SO_4, and steam concentrated to a 10 ml hexane solution. Sonification of the clay pellet and soxhlet extraction did not increase the recovery of Kelthane® from clay. The same gas chromatographic analysis procedure was used as described previously. Recovery of spiked samples averaged 102.4% (n = 4, sd = 2.1); variation between duplicates averaged 5.1% (n = 8, sd = 4.2). Similar results were obtained with packed and capillary GC columns.

Statistical Methods

Bioconcentration factor data from the study were fitted to a three-way analysis of variance (ANOVA) fixed effects model. The data were logarithm transformed to better conform with the assumptions of this model, especially normality and homoscedasticity. The original data set was not homoscedastic since the variance increased with the mean; transformation helped alleviate this problem.

Means were compared by using Scheffe's method of multiple comparisons to obtain 95% confidence intervals for the differences in means. This method gives simultaneous 100 (1-α)% confidence intervals for comparisons for a *post hoc* analysis of the data. Similar results were obtained using the related Bonferroni method of multiple comparisons (a more rigorous test used when a smaller number of pre-selected comparisons is specified).

Results

Mean bioconcentration factors (BCF) for clay and Kelthane® concentration exposures at five sampling times are presented in Table 1. For a given concentration and day, fish in water without clay had a 35% higher BCF than the fish in water with clay (95% confidence interval (ci) 19% to 53%). Fish in the low Kelthane® concentration had a 10% higher BCF than fish in the high concentration (95% ci 3% to 24%). Results of the three-way analysis of variance (Table 2) indicated that clay and sampling day had a highly significant effect on bioconcentration and that Kelthane® concentration had a barely significant effect. There were no significant interaction effects. Exposure conditions appeared to be suitable, because no adverse effects on test fish were observed during the experiment. Mean weights of fish increased from 0.2 to 0.7 g over the 28 days and were not different (p = 0.05) between treatments.

Bioconcentration was modeled by a two-compartment (water/fish), first-order diffusion equation:

$$\frac{dC_T}{dt} = K_1C_w - K_2C_T$$

Model parameters (K_1, K_2) were estimated for each data set by least-squares, non-linear regression analysis, employing the logarithm transformation of the solution to the above differential equation:

$$Y = \log \frac{C_T}{C_w} = \log\left(\frac{K_1}{K_2}(1 - e^{-K_2t})\right) + e$$

where

C_T = fish tissue Kelthane® concentration
C_w = water Kelthane® concentration
K_1 = Kelthane® uptake rate
K_2 = Kelthane® depuration rate
t = time of exposure
e = error; the errors are assumed indepen-

Table 2. Three-way (Kelthane® concentration, clay prevalence and sampling day) ANOVA results for experimentally determined bioconcentration factors[a]

Source	Sum of squares	D.F.	Mean square	f	Tail probability
Mean BCF	490.9951	1	490.9951	171405.7	<0.00005
Concentration (high or low)	0.0144	1	0.0144	5.0	0.04
Clay (present or absent)	0.1515	1	0.1515	52.9	<0.00005
Sampling day	3.6588	4	0.9147	319.3	<0.00005
Conc. × Clay	0.0012	1	0.0012	0.4	0.52
Conc. × Day	0.0110	4	0.0028	1.0	0.45
Clay × Day	0.0035	4	0.0009	0.3	0.87
Conc. × Clay × Day	0.0116	4	0.0029	1.0	0.42
Error	0.0516	18	0.0029		

[a] Unrounded bioconcentration factor numbers were used in ANOVA calculations

Table 3. Values estimated from regression analysis of 28-day uptake data

	High Kelthane® level		Low Kelthane® level	
	No clay	Clay	No clay	Clay
Uptake rate (K_1, $day^{-1} \times 10^{-3}$)	0.96	0.73	0.95	0.73
Depuration rate (K_2, day^{-1})	0.064	0.076	0.050	0.050
Steady-state BCF	15000	9500	18900	14500
R^2 (%)	99.0	98.5	99.2	99.7
Time to 95% of steady-state (days)	47	39	60	60

dently and normally distributed with mean zero and constant variance σ^2.

Values calculated for the rate functions K_1 and K_2 obtained from individual regressions for each exposure condition (Table 3) suggest that the highly significant effect of clay on BCF was best related to the uptake differences in the presence and absence of clay, and that the marginally significant effect of concentration on BCF was best related to differences in depuration. However, this interpretation is tentative. The appropriateness of the model cannot be definitively tested with the available data because the quantity and temporal range cause K_1 and K_2 to be correlated. Calculated steady-state BCFs ranged from 9,500 to 18,900. Estimated times to 95% of steady-state ranged from 39 to 60 days.

In the sorption experiment, a mean 30.2% of the Kelthane® present at a total measured concentration of 14.7 μg/L was found sorbed to the centrifuged (22,000 rpm) clay. The percentages at two and four times higher Kelthane® concentrations were similar (34.3 and 22.1%, respectively). For all sorption analyses, 95.0% (sd = 5.4, n = 8) of the total Kelthane® measured in whole samples (uncentrifuged) was accounted for by adding the amounts measured in clay and supernatant.

Discussion

The BCFs for Kelthane® in fathead minnows are similar to but generally higher than values one would predict from physical data for this pesticide. The solubility for Kelthane® in Lake Superior water without clay under test conditions was determined to be 590 μg/L (sd = 55, n = 4). Chiou *et al.* (1977) derived a linear regression equation (R^2 = 0.930) for relating water solubility to bioconcentration in rainbow trout for a "selected" series of seven chlorinated organic compounds. Using their equation and the solubility value from the present study, a bioconcentration factor of 2,000 would be calculated, which compares reasonably well with the empirical data from this study. Previous bioconcentration tests in this laboratory have indicated that fathead minnows accumulate higher residues than rainbow trout exposed to the same chemicals. Applying the 0.5 log BCF rainbow-to-fathead accommodation factor used previously by Veith *et al.* (1979) to the regression equation, a calculated BCF of 6,400 is obtained. This substantially improves the estimate.

The partitioning of an organic chemical between *n*-octanol and water in a 2-phase system is also used

to estimate bioconcentration factors. The ratio of the equilibrium concentrations of the partitioning are expressed as P (or alternatively, as Kow). Measurements of BCF and P for 122 organic chemicals have been obtained to establish a regression equation relating BCF to the partition coefficient P (Veith and Kosian 1982). Employing this equation (log BCF = 0.79 log P − 0.40, R^2 = 0.86), and a log P for Kelthane® of 5.56[1], a BCF of 9,800 is calculated. This is closer to our fish exposure values than the one derived from solubility data, although they are both close enough to support further the utility of these indirect estimating techniques for screening chemicals for bioconcentration potential. Applying formulas cited by ASTM (1981) for estimating time to steady-state using log P and water solubility, Kelthane® values are obtained of 19.4 and 20.2 days, respectively, or less than half the estimated time to 95% of steady-state from our exposure data and model.

Statistical evidence indicated a slight effect of Kelthane® concentration on BCF. It is clear (see Table 1) that the practical significance of such BCF differences is very small compared to the magnitude of the water concentration range tested. Clay, on the other hand, had a definite effect on BCF, the 35% reduction appearing consistent with the 30.2% sorption of total Kelthane® by clay at 14.7 µg/L. An additional small amount of Kelthane® associated with particles not removed by centrifugation was measured as part of the supernatant fraction, so the actual amount sorbed was somewhat higher than 30.2%. These results reinforce the presumption that Kelthane® is not desorbed from the clay as it becomes more concentrated during centrifugation. Because no consistent relationship could be discerned between percent sorption and Kelthane® at higher concentrations, it is presumed that a similar degree of sorption occurred at a low exposure concentration. Detection limit constraints prevented measurement of this quantity.

A factor of 4,100 was calculated for the bioconcentration of Kelthane® on clay at a water concentration of 14.7 µg/L. The influence of organic content on sorption by soils of non-polar organic chemicals has been reported and reviewed by many investigators (Chiou *et al.* 1979; Kenaga 1980; Kenaga and Goring 1980). Kenaga and Goring (1980) presented formulas relating the three values—bioconcentration factor, water solubility, and soil sorption in terms of organic carbon content—to one another. Using their organic carbon-water solubility equation (log K_{oc} = 3.64 − 0.55 (log WS) ± 1.23 orders of magnitude 95% ci), an estimated soil organic matter sorption coefficient (accumulation factor) of 5,800 is calculated for Kelthane® from its measured solubility (590 µg/L). In the present study, the organic carbon content of the clay was determined to be less than 1% (the detection limit for analysis) so an accumulation of greater than 400,000 times the water concentration by the organic fraction would be necessary to account for the Kelthane® level measured on the clay. This concentration factor falls well beyond the 95% confidence interval (470 to 71,000 times the water concentration) given for the equation and indicates a substantial sorptive capacity of the inorganic fraction of the clay. A subsequent test with the organic fraction more precisely quantified or eliminated would be instructive. The importance of the particle size-surface area relationship is indicated by the finding that the 20% of the particulates remaining in suspension at 2,600 rpm centrifugation contained over half of the sorbed Kelthane® (50.1% of that found in sediments removed by 22,000 rpm).

The bioconcentration of the *p,p'* isomer to a greater degree than *o,p* isomer was interesting. Both isomers were equally soluble so the difference was not due to availability of *o,p* in the water. If BCF were calculated on the basis of total Kelthane® they would be somewhat smaller (about 20%) and, for analytical reasons, more variable. Similar factors would probably also be obtained using radio-labeled Kelthane to determine water and tissue concentrations unless care was taken to differentiate between identically marked isomers.

Our results indicating that Kelthane® sorbed onto clay was not bioconcentrated are in accord with the observations by Lockhart (1980) that river water with a suspended solids concentration of 75 mg/L reduced tissue accumulation of methoxychlor, another chlorinated hydrocarbon pesticide, in fish to about 50% of that resulting from exposure to laboratory water containing <1 mg/L of suspended solids. Similarly, Sebastien and Lockhart (1981) found accumulation of methoxychlor by rainbow trout in laboratory test water containing a particulate-coated formulation to be less than 20% of the accumulation in water containing an emulsifiable-concentrate formulation. Some investigators have observed greater accumulation in test systems where organisms are allowed access to a contaminated sediment which also serves as the water source of test chemical. Fathead minnows had 6 times higher PCB residues (Halter and Johnson 1977) and amphipods had 2 to 11 times higher hexachlorobiphenyl residues (Lynch and Johnson 1982) than did organisms in test chambers where they were screened from the substrates. These authors have hypothesized the increased uptake to be a two-stage process in which elevated water concentrations of

[1] Log P calculated by the fragment method described by Hansch and Leo

the chemical occur near the water-particulate interface first and secondarily produce higher accumulation rates. On the other hand, Southworth *et al.* (1979) observed no significant difference in tissue concentrations of acridine, a much less bioaccumulative compound than PCBs or hexachlorobiphenyl, in sediment-exposed and -unexposed fathead minnows. In either case, the water concentration of chemical appears to be the factor controlling accumulation of residues.

In summary, bioconcentration testing was demonstrated to be a means of quantitatively examining the relationship between biological availability and chemical/medium interactions for the chlorinated hydrocarbon pesticide Kelthane®. The degree of reduction in bioconcentration was about what one would expect if assuming that sorbed Kelthane® was biologically unavailable to the fish. However, it may not be appropriate to use the organic content of suspended particulates, at least where it is very low, as a basis for indirectly estimating the extent of an effect on bioconcentration. Further tests are needed relating the chemical and physical interaction of organic chemicals with important environmental components, to responses such as bioconcentration or mortality. Sufficient understanding of these relationships will lead to improvement in predictive capabilities and reduce the cost of hazard evaluation or organic chemicals.

Acknowledgments. We thank Ms. Chris Stidley and Dr. Russell Erickson for conducting ANOVA and regression evaluations of data; Dr. Philip Cook for consultation related to geochemistry; Mr. Frank Puglisi for assistance in developing the exposure system; Mr. David Marklund for clay filtration and weighing; and the Rohm and Haas Company for supplying the technical grade Kelthane® used in the experiment.

References

American Public Health Association: Standard methods for the examination of water and wastewater. 14 ed. New York:American Public Health Association (1974).

American Society for Testing and Materials: Proposed standard practice for conducting bioconcentration tests with fishes and saltwater bivalve molluscs. Draft manuscript #3, June 10, Duluth, Minnesota (1981).

Benoit, D. A., and F. A. Puglisi: A simplified flow-splitting chamber and siphon for proportional diluters. Water Res. **7**, 1915 (1973).

Brungs, W. A., and G. W. Bailey: Influence of suspended solids on the acute toxicity of endrin to fathead minnows. Proc. 21st. Purdue Indust. Waste Conf., Lafayette, Part I, 50, 4 (1967).

Chiou, C. T., V. H. Freed, D. W. Schmeeding, and R. L. Kohnert: Partition coefficient and bioconcentration of selected organic chemicals. Environ. Sci. Technol. **11**, 475 (1977).

Chiou, C. T., L. J. Peters, and V. H. Freed: A physical concept of soil-water equilibria for non-ionic organic compounds. Science **206**, 831 (1979).

Committee on Methods for Toxicity Tests with Aquatic Organisms: Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. U.S. Environmental Protection Agency, Duluth, MN EPA-660/3-75-009 (1975).

Ferguson, D. E., J. L. Ludke, J. P. Wood, and J. W. Prather: The effects of mud on the bioactivity of pesticides on fishes. J. Mississippi Acad. Sci. **11**, 219 (1965).

Halter, M. T., and H. E. Johnson: A model system to study the desorption and biological availability of PCB in hydrosoils. In F. L. Mayer, and J. F. Hamelink (eds.): Aquatic toxicology and hazard assessment, p. 178, ASTM, STP 634. Philadelphia: American Society for Testing and Materials (1976).

Hansch, C., and A. J. Leo: Substituent constants for correlation analysis in chemistry and biology. New York: John Wiley & Sons (1979).

Holcombe, G. W., G. L. Phipps, and D. K. Tanner: The acute toxicity of Kelthane®, Dursban®, disulfoton, pydrin, and permethrin to fathead minnows (*Pimephales promelas*) and rainbow trout (*Salmo gairdneri*). Environ. Pollut. (Series A) **29**, 167 (1982).

Jackson, M. F.: Soil chemical analysis—advanced course. Monograph 115, Chapter 3, p. 101. College of Agriculture, Dept. of Soils, University of Wisconsin, Madison. (1956).

Kenaga, E. E.: Predicted bioconcentration factors and soil sorption coefficients of pesticides and other chemicals. Ecotoxicol. Environ. Safety **4**, 26 (1980).

Kenaga, E. E., and C. A. I. Goring: Relationship between water solubility, soil sorption, octanol-water partitioning, and concentration of chemicals in biota. In J. G. Eaton, P. R. Parrish, and A. C. Hendricks (eds.): Aquatic toxicology, p. 78, ASTM, STP 707. Philadelphia: American Society for Testing and Materials (1980).

Lockhart, W. L.: Methoxychlor studies with fish: Athabaska River exposures and experimental exposures. In W. O. Haufe, and G. C. R. Croome (eds.): Control of black flies in the Athabaska River, p. 183. Edmonton: Technical Report Alberta Environment (1980).

Lyncl., T. R., and H. E. Johnson: Availability of a hexachlorobiphenyl isomer to benthic amphipods from experimentally contaminated natural sediments. In J. G. Pearson, R. B Foster, and W. E. Bishop (eds.): Aquatic toxicology and hazard assessment, p. 273, ASTM, STP 766. Philadelphia: American Society for Testing and Materials (1982).

Marklund, D., P. Morton, S. Kohlbry, and E. Ruenger: Results of analyses performed under EPA task plans NAARD0309 and NAARD0308. Lake Superior Basin Studies Center Manuscript, University of Minnesota-Duluth, November 1981.

Mount, D. I., and W. A. Brungs: A simplified dosing apparatus for toxicity studies. Water Res. **1**, 21 (1967).

Phipps, G. L., G. W. Holcombe, and J. T. Fiandt: A saturator system for generating toxic water solutions for toxicity tests. Prog. Fish-Cult. **44**, 115 (1982).

Sebastien, R. J., and W. L. Lockhart: The influence of formulation on toxicity and availability of a pesticide (methoxychlor) to black fly larvae (Diptera: Simuliidae), some nontarget insects and fish. Can. Entomol. **13**, 281 (1981).

Southworth, G. R., B. R. Parkhurst, and J. J. Beauchamp: Accumulation of acridine from water, food, and sediment by the fathead minnow, *Pimephales promelas*. Water, Air, and Soil Pollut. **12**, 331 (1979).

Spehar, R. L., D. K. Tanner, and J. H. Gibson: The effects of Kelthane® and pydrin on early life stages of fathead minnows (*Pimephales promelas*) and amphipods (*Hyalella azteca*). In J. G. Pearson, R. B. Foster, and W. E. Bishop (eds.): Aquatic toxicology and hazard assessment, p. 234, ASTM, STP 766. Philadelphia: American Society for Testing and Materials (1982).

Veith, G. D., and P. Kosian: Estimating bioconcentration potential from octanol/water partition coefficients. In D. Mackay, S. Paterson, S. J. Eisenreich, and M. S. Simmons (eds.): The physical behavior of PCBs in the Great Lakes, p. 269. Ann Arbor: Ann Arbor Science (1982).

Veith, G. D., D. L. DeFoe, and B. V. Bergstedt: Measuring and estimating the bioconcentration factor of chemicals in fish. J. Fish. Res. Board Can. **36**, 1040 (1979).

Received for publication October 12, 1982 and in revised form January 24, 1983.